

Usefulness of *hsp60* Gene for Identification and Classification of Gram-negative Anaerobic Rods

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Abstract:

Gram-negative anaerobic rods are part of the indigenous microbiota of human and animal gastrointestinal tract, oral cavity, etc. The taxonomy of Gram-negative anaerobic rods has undergone significant changes in the past decade. The recent taxonomic changes of Gram-negative anaerobic rods are mainly based on the 16S rRNA gene sequence analysis. However, accurate identification of an isolate closely related to known species is often difficult only with the 16S rRNA gene analysis and DNA-DNA hybridization (DDH) is required for differentiating closely related species. Because DDH method is time-consuming and laborious, an alternative molecular marker is anticipated. The *hsp60* gene has been shown to be more discriminative than the 16S rRNA gene for the identification of some *Prevotella* species. More recently, the *hsp60* gene has been found to be an alternative phylogenetic marker for the classification of species of the genus *Bacteroides*. Except for the genus *Bacteroides*, however, there is little information whether the *hsp60* gene is a suitable phylogenetic marker for the taxonomy of Gram-negative anaerobic rods. In this study, we evaluated the usefulness of *hsp60* gene for the identification and classification of a broad range of Gram-negative anaerobic rods, including the genera *Barnesiella*, *Butyricimonas*, *Odoribacter*, *Parabacteroides*, *Paraprevotella*, *Porphyromonas*, *Prevotella*, *Tannerella* as well as *Bacteroides*. The *hsp60* gene sequences (558 bp) were determined for 121 strains of Gram-negative anaerobic rods. The mean pairwise *hsp60* gene sequence similarity (73.8–97.1%) between species in each genus was significantly less than that of the 16S rRNA gene sequence (88.3–96.3%). Each genus formed a distinct clade in the phylogenetic analysis of the *hsp60* gene sequence as well as the 16S rRNA gene sequence. The phylogenetic analysis indicated a higher evolutionary rate for the *hsp60* gene sequence than the 16S rRNA gene sequence, especially in the genera *Porphyromonas* and *Prevotella*. This study suggests that the *hsp60* gene is useful alternative phylogenetic marker for the identification and classification of a broad range of Gram-negative anaerobic rods.

Key words: *hsp60* gene, Gram-negative anaerobic rods, identification, classification